Mutator Genotypes Always Rise to Fixation in *Pseudomonas aeruginosa* Biofilm Populations. Katrina Harris[1] and Vaughn Cooper[1], [1]University of Pittsburgh, 450 Technology Drive, Pittsburgh, PA 15219

Most bacterial growth occurs on surfaces in structures known as biofilms. Biofilm growth involves the production of polysaccharides and other factors that promote adherence and provide protection. Structured growth introduces nutrient gradients into the population that the cells must find a way to tolerate. Evolutionary dynamics in biofilms may be fundamentally different: 1) interactions among cells leading to multicellular phenotypes 2) increased frequency of mutator genotypes, which lead to a higher genome wide mutation rate, observed in nature and infections are seen more frequently in biofilm populations than in planktonic populations. Here we evolved the pathogen *Pseudomonas aeruginosa* (PA) for 600 generations under the biofilm lifecycle of attachment, growth, and reattachment daily, and the planktonic lifecycle where the cells grow free floating in liquid medium. Mutator genotypes evolve and rise to fixation in all biofilm populations, reflected in the increased genotypic and phenotypic diversity of these populations, but are never detected in planktonic populations. Short term evolution studies of mutator and non-mutator genotype mixed populations have been performed to determine the required mutational supply for mutator rise and fixation. Clonal and population analysis of these populations reveal that mutators can invade populations at lower starting concentrations in biofilm growth compared to planktonic growth, meaning a smaller mutational supply is required for the rise of the mutator phenotype in the biofilm populations. These results could be due to the smaller total size of a biofilm population, may be a result of the biofilm population not being one homozygous mixture but being made up of smaller distinct subpopulations or may even be because the ancestor was more adapted to the biofilm environment at the start of the experiment. Studying the cellular interactions of these bacterial biofilm populations, how diverse yet robust PA biofilms contain mutator genotypes that increase variation within the population, may give insights into early multicellular interactions.